Comparison of TACE catalytic domain structures in TACE-inhibitor complexes with TACE structure in TACE-TAPI complex:

1. **Molecule A of TACE-TAPI complex structure (four molecules per asymmetric unit) is used as template;**
2. **Active-site residues including residues 346-352, 396-412, 415, 435-441;**
3. **Molecule 1 in TACE-inhibitor dimer structures are used for the comparisons with TACE-TAPI molecule A.**
4. **The numbers shown below are RMS difference between equivalent atoms from both structures in Å/atom.**
5. **m-c: main-chain**

Inhibitor	all monomer Cα	Act. Site Cα	Act. Site m-c	Act. Site all atoms
3	0.844	0.266	0.28	0.43
4	0.816	0.34	0.34	0.41
5	0.876	0.354	0.37	0.45
6	0.696	0.314	0.32	0.37
7	0.902	0.372	0.38	0.50
8	0.844	0.356	0.36	0.50
9	0.872	0.39	0.40	0.51
10	0.87	0.296	0.31	0.41
11	0.85	0.302	0.32	0.40
12	0.706	0.276	0.28	0.36
13	0.718	0.32	0.34	0.42
14	0.724	0.338	0.34	0.41
15	0.728	0.386	0.39	0.42
16	0.734	0.274	0.29	0.36

Docking Algorithms

1.) Proteins 1998 Nov 15;33(3):367-82

Flexible docking using Tabu search and an empirical estimate of binding affinity.

Baxter CA, Murray CW, Clark DE, Westhead DR, Eldridge MD

Proteus Molecular Design Ltd., Macclesfield, Cheshire, United Kingdom.

This article describes the implementation of a new docking approach. The method uses a Tabu search methodology to dock flexibly ligand molecules into rigid receptor structures. It uses an empirical objective function with a small number of physically based terms derived from fitting experimental binding affinities for crystallographic complexes. This means that docking energies produced by the searching algorithm provide direct estimates of the binding affinities of the ligands. The method has been tested on 50 ligand-receptor complexes for which the experimental binding affinity and binding geometry are known. All water molecules are removed from the structures and ligand molecules are minimized in vacuo before docking. The lowest energy geometry produced by the docking protocol is within 1.5 A root-mean square of the experimental binding mode for 86% of the complexes. The lowest energies produced by the docking are in fair agreement with the known free energies of binding for the ligands.

PMID: 9829696

2.) Protein Sci 1998 Apr;7(4):938-50

Flexible ligand docking using conformational ensembles.

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Molecular docking algorithms suggest possible structures for molecular complexes. They are used to model biological function and to discover potential ligands. A present challenge for docking algorithms is the treatment of molecular flexibility. Here, the rigid body program, DOCK, is modified to allow it to rapidly fit multiple conformations of ligands. Conformations of a given molecule are pre-calculated in the same frame of reference, so that each conformer shares a common rigid fragment with all other conformations. The ligand conformers are then docked together, as an ensemble, into a receptor binding site. This takes advantage of the redundancy present in differing conformers of the same molecule. The algorithm was tested using three organic ligand protein systems and two protein-protein systems. Both the bound and

unbound conformations of the receptors were used. The ligand ensemble method found conformations that resembled those determined in X-ray crystal structures (RMS values typically less than 1.5 A). To test the method's usefulness for inhibitor discovery, multi-compound and multi-conformer databases were screened for compounds known to bind to dihydrofolate reductase and compounds known to bind to thymidylate synthase. In both cases, known inhibitors and substrates were identified in conformations resembling those observed experimentally. The ligand ensemble method was 100-fold faster than docking a single conformation at a time and was able to screen a database of over 34 million conformations from 117,000 molecules in one to four CPU days on a workstation.

PMID: 9568900

3.) J Comput Aided Mol Des 1997 Jul;11(4):333-44

QXP: powerful, rapid computer algorithms for structure-based drug design.

McMartin C, Bohacek RS

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New methods for docking, template fitting and building pseudo-receptors are described. Full conformational searches are carried out for flexible cyclic and acyclic molecules. QXP (quick explore) search algorithms are derived from the method of Monte Carlo perturbation with energy minimization in Cartesian space. An additional fast search step is introduced between the initial perturbation and energy minimization. The fast search produces approximate low-energy structures, which are likely to minimize to a low energy. For template fitting, QXP uses a superposition force field which automatically assigns short-range attractive forces to similar atoms in different molecules. The docking algorithms were evaluated using X-ray data for 12 protein-ligand complexes. The ligands had up to 24 rotatable bonds and ranged from highly polar to mostly nonpolar. Docking searches of the randomly disordered ligands gave rms differences between the lowest energy docked structure and the energy-minimized X-ray structure, of less than 0.76 A for 10 of the ligands. For all the ligands, the rms difference between the energy-minimized X-ray structure and the closest docked structure was less than 0.4 A, when parts of one of the molecules which are in the solvent were excluded from the rms calculation. Template fitting was tested using four ACE inhibitors. Three ACE templates have been previously published. A single run using QXP generated a series of templates which contained examples of each of the three. A pseudo-receptor, complementary to an ACE template, was built out of small molecules, such as pyrrole, cyclopentanone and propane. When individually energy minimized in the pseudo-receptor, each of the four ACE inhibitors moved with an rms of less than 0.25 A. After random perturbation, the inhibitors were docked into the

pseudo-receptor. Each lowest energy docked structure matched the energy-minimized geometry with an rms of less than 0.08 A. Thus, the pseudo-receptor shows steric and chemical complementarity to all four molecules. The QXP program is reliable, easy to use and sufficiently rapid for routine application in structure-based drug design.

PMID: 9334900

4.) J Mol Biol 1997 Apr 4;267(3):727-48

Development and validation of a genetic algorithm for flexible docking.

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Department of Information Studies and Krebs Institute for Biomolecular Research, University of Sheffield, Western Bank, UK.

Prediction of small molecule binding modes to macromolecules of known three-dimensional structure is a problem of paramount importance in rational drug design (the "docking"problem). We report the development and validation of the program GOLD (Genetic Optimisation for Ligand Docking). GOLD is an automated ligand docking program that uses a genetic algorithm to explore the full range of ligand conformational flexibility with partial flexibility of the protein, and satisfies the fundamental requirement that the ligand must displace loosely bound water on binding. Numerous enhancements and modifications have been applied to the original technique resulting in a substantial increase in the reliability and the applicability of the algorithm. The advanced algorithm has been tested on a dataset of 100 complexes extracted from the Brookhaven Protein DataBank. When used to dock the ligand back into the binding site, GOLD achieved a 71% success rate in identifying the experimental binding mode.

PMID: 9126849

5.) Chem Biol 1997 Apr;4(4):297-307

Structure-based design and combinatorial chemistry yield low nanomolar inhibitors of cathepsin D.

Kick EK, Roe DC, Skillman AG, Liu G, Ewing TJ, Sun Y, Kuntz ID, Ellman JA

Department of Chemistry University of California Berkeley, CA 94720-4160, USA.

BACKGROUND: The identification of potent small molecule ligands to receptors and enzymes is one of the major goals of chemical and biological research. Two powerful

new tools that can be used in these efforts are combinatorial chemistry and structure-based design. Here we address how to join these methods in a design protocol that produces libraries of compounds that are directed against specific macromolecular targets. The aspartyl class of proteases, which is involved in numerous biological processes, was chosen to demonstrate this effective procedure. RESULTS: Using cathepsin D, a prototypical aspartyl protease, a number of low nanomolar inhibitors were rapidly identified. Although cathepsin D is implicated in a number of therapeutically relevant processes, potent nonpeptide inhibitors have not been reported previously. The libraries, synthesized on solid support, displayed nonpeptide functionality about the (hydroxyethyl)amine isostere. The (hydroxyethyl)amine isostere, which targets the aspartyl
protease class, is a stable mimetic of the tetrahedral intermediate of amide hydrolysis. Structure-based design, using the crystal structure of cathepsin D complexed with the peptide-based natural product pepstatin, was used to select the building blocks for the library synthesis. The library yielded a 'hit rate' of 6-7% at 1 microM inhibitor concentrations, with the most potent compound having a Ki value of 73 nM. More potent, nonpeptide inhibitors (Ki = 9-15 nM) of cathepsin D were rapidly identified by synthesizing and screening a small second generation library. CONCLUSIONS: The success of these studies clearly demonstrates the power of coupling the complementary methods of combinatorial chemistry and structure-based design. We anticipate that the general approaches described here will be successful for other members of the aspartyl protease class and for many other enzyme classes.

PMID: 9195867

6.) J Mol Biol 1996 Aug 23;261(3):470-89

A fast flexible docking method using an incremental construction algorithm.

Rarey M, Kramer B, Lengauer T, Klebe G

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We present an automatic method for docking organic ligands into protein binding sites. The method can be used in the design process of specific protein ligands. It combines an
appropriate model of the physico-chemical properties of the docked molecules with efficient methods for sampling the conformational space of the ligand. If the ligand is flexible, it can adopt a large variety of different conformations. Each such minimum in conformational space presents a potential candidate for the conformation of the ligand in the complexed state. Our docking method samples the conformation space of the ligand on the basis of a discrete model and uses a tree-search technique for placing the ligand incrementally into the active site. For placing the first fragment of the ligand

into the protein, we use hashing techniques adapted from computer vision. The incremental construction algorithm is based on a greedy strategy combined with efficient methods for overlap detection and for the search of new interactions. We present results on 19 complexes of which the binding geometry has been crystallographically determined. All considered ligands are docked in at most three minutes on a current workstation. The experimentally observed binding mode of the ligand is reproduced with 0.5 to 1.2 A rms deviation. It is almost always found among the highest-ranking conformations computed.

PMID: 8780787

7.) Chem Biol 1996 Jun;3(6):449-62

Hammerhead: fast, fully automated docking of flexible ligands to protein binding sites.

Welch W, Ruppert J, Jain AN

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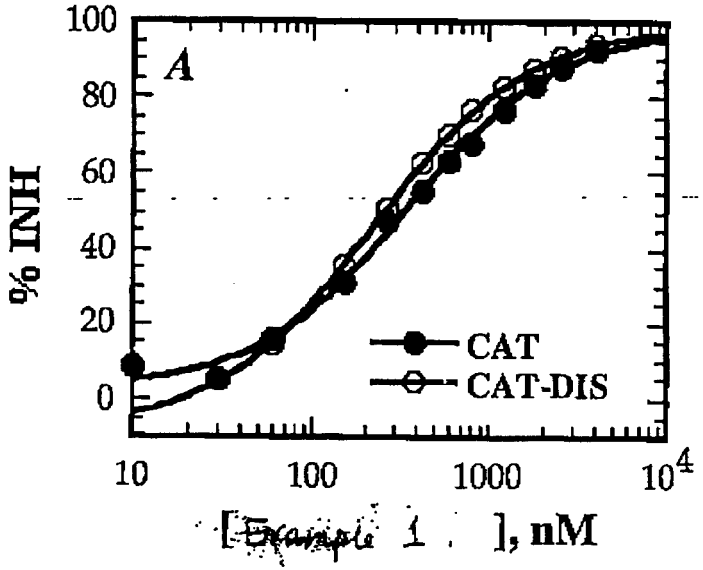
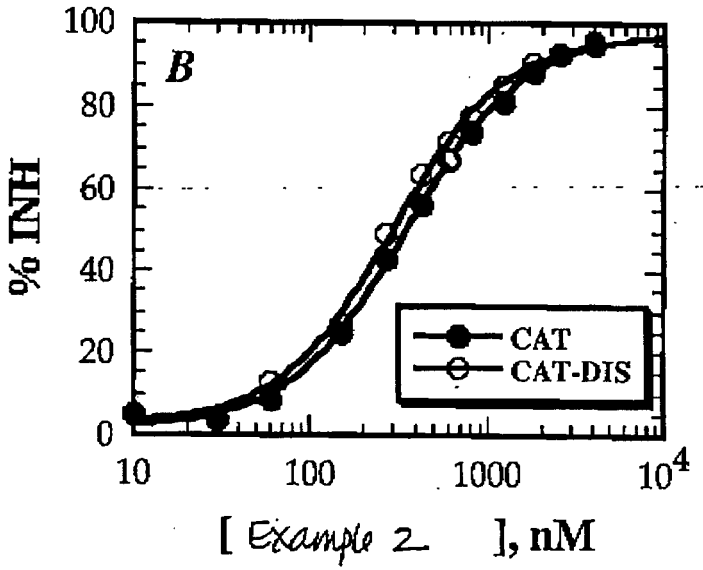
BACKGROUND: Molecular docking seeks to predict the geometry and affinity of the binding of a small molecule to a given protein of known structure. Rigid docking has long been used to screen databases of small molecules, because docking techniques that account for ligand flexibility have either been too slow or have required significant human intervention. Here we describe a docking algorithm, Hammerhead, which is a fast, automated tool to screen for the binding of flexible molecules to protein binding sites. RESULTS: We used Hammerhead to successfully dock a variety of positive control ligands into their cognate proteins. The empirically tuned scoring function of the algorithm predicted binding affinities within 1.3 log units of the known affinities for these ligands. Conformations and alignments close to those determined crystallographically received the highest scores. We screened 80 000 compounds for binding to streptavidin, and biotin was predicted as the top-scoring ligand, with other known ligands included among the highest-scoring dockings. The screen ran in a few days on commonly available hardware. CONCLUSIONS: Hammerhead is suitable for screening large databases of flexible molecules for binding to a protein of known structure. It correctly docks a variety of known flexible ligands, and it spends an average of only a few seconds on each compound
during a screen. The approach is completely automated, from the elucidation of protein binding sites, through the docking of molecules, to the final selection of compounds for assay.

PMID: 8807875

Comparison of IC_{50} Values of Example 1 and Example 2 against Cat and Cat-Dis TACE

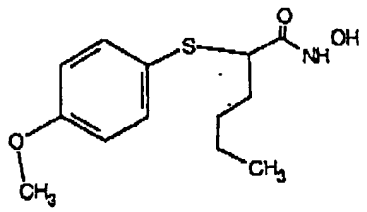




Compounds	IC50, nM	
	Cat	Cat-Dis
Example 1	344 ± 38	217 ± 9
Example 2	346 ± 13	291 ± 21

Table 1

EX 1



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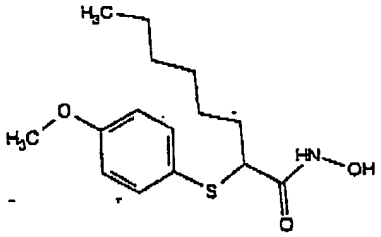

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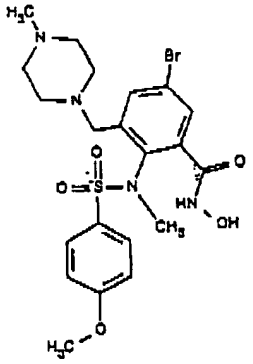

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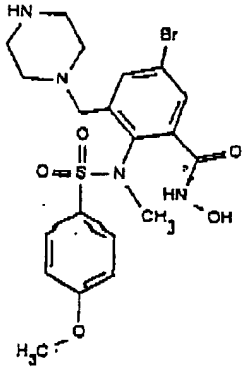

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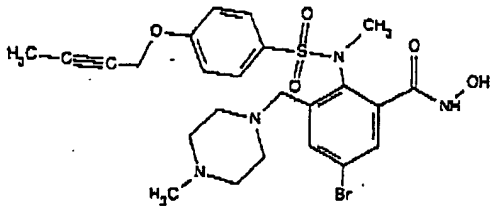

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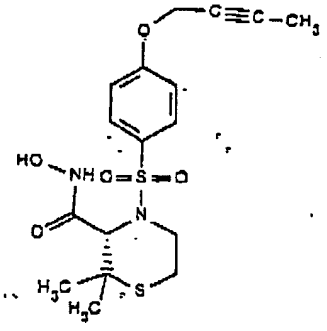

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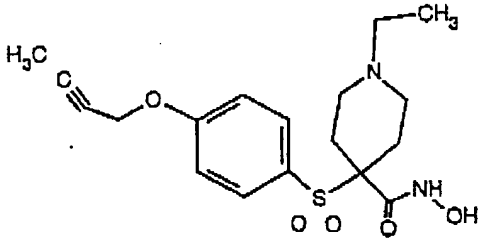

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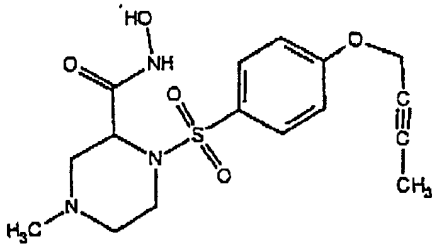

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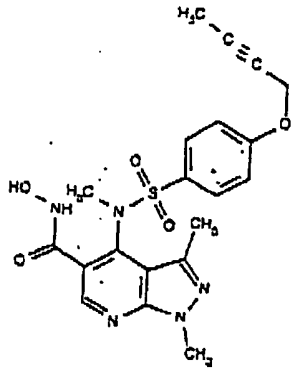

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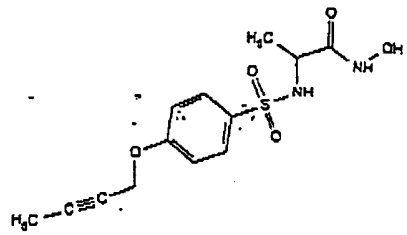

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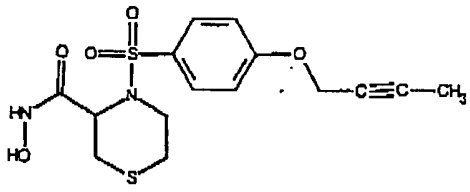

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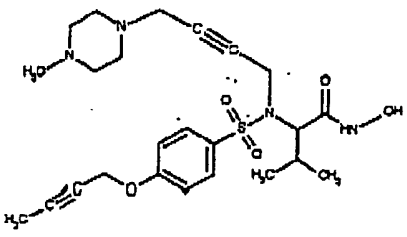

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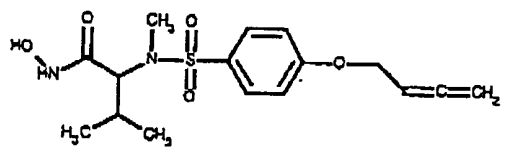

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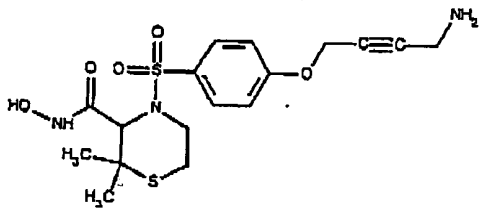

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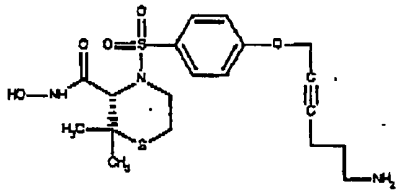

Ex 16